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SHORT COMMUNICATION
Prevalence of feline coronavirus antibodies in cats in Bursa province, Turkey, by an enzyme-linked immunosorbent assay

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Feline sera from Bursa province (Turkey) were assayed for coronavirus antibody using an enzyme-linked immunosorbent assay (ELISA). The study was performed on 100 sera collected from cats belonging to catteries or community shelters and to households. The serum samples were initially tested with the virus neutralisation (VN) test and the results were then compared with the ELISA. The VN yielded 79 negative and 21 positive sera but the ELISA confirmed only 74 as negative. The ELISA-negative sera were also found to be free of feline coronoviruses-specific antibodies by Western blotting. Using the VN as the gold standard test, ELISA had a sensitivity of 100% and a specificity of 93.6%, with an overall agreement of 95%. The Kappa (κ) test indicated high association between the two tests ($\kappa = 0.86$, 95% confidence interval (CI) 0.743–0.980). The positive predictive value (PPV) was 0.8, and the negative predictive value (NPV) was 0.93. The prevalence of FCoV II antibodies in the sampled population based on the gold standard was 62% (95% CI 0.44–0.77) among multi-cat environments, and 4% (95% CI 0.01–0.11) among single cat households.

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The coronaviruses are a closely related family of viruses responsible for severe diseases of the upper respiratory tract, enteritis, serositis, hepatitis and encephalitis in avian and mammalian species. They are large, enveloped, positive, single-stranded RNA viruses with the largest genomes among all RNA viruses. Most of the 5′ two-thirds of the genome is occupied by the pol gene, while the 3′ one-third consists of genes encoding the structural proteins S, E, M and N as well as non-structural proteins.[1] Feline coronaviruses (FCoVs) are divided into two biological types: an enteritis-inducing coronavirus (FECV) and the systemic pathogen infectious peritonitis virus (FIPV). In regard to genetic and antigenic properties, however, FECV and FIPV are virtually the same virus and it is possible to distinguish an isolate regardless the clinical signs exhibited by the host, only after biomolecular examinations. It is believed that the avirulent FECVs remain confined to the digestive tract and subclinically infected cats may spread the infection to susceptible kittens via the fecal-oral route over a period of months.[2–4] A recent study, therefore, showed that systemic infection with FCoV was not a key events in pathogenesis of FIP. Kipar et al[5] demonstrated that all cats with FIP and the vast majority of FCoV-infected cats without FIP exhibited systemic infection, but the viral load in the haemolymphatic tissues of cats with FIP was generally higher than in FCoV-infected cats without disease. Mutation events in some of them could lead to the generation of the virulent FIPV that would leave the gut and generalise via infected monocytes. These animals develop fatal FIP.[6] There are two distinct serotypes of FCoV, I and II, segregated by virus neutralisation assay (VN).[7–9] Molecular analysis confirmed that FCoV II originated from RNA recombination events during which the spike gene of canine coronavirus was incorporated into FCoV I genome.[3,8] In the field, the prevalence of FCoV I seems to be higher, as serotype II accounts for only 20–30% of all FCoV infections.[9] Therefore, a recent study performed in southern Italy

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revealed a distribution of the serotypes which is slightly different from the patterns reported.[10] The laboratory diagnosis and control of FIP is seriously hampered by the failure of most FCoV to replicate in cell cultures.[6] This observation led to the evaluation of anti-FCoV antibodies as an useful method to monitor infection especially in breeding catteries where FCoVs infection is widely spread. Although there are clinical cases of FIP in Turkey, no study has been reported on the distribution of FCoV antibodies in the Turkish cat population. Thus the aim of this study was to create a preliminary data on the seroprevalence of FCoV in Turkey.

One hundred sera, collected randomly from cats belonging to catteries, community shelters and households from Bursa province, were tested. At the time of sampling all the cats were older than 1 year of age. Seventy-one sera were collected from household cats that were not in contact with other cats. Twenty-seven cats ranged from 1 to 4 years old, 15 cats aged from 4 to 9 years old, and the remaining 29 cats were older than 10 years. The other 29 sera were from cats that lived in different catteries near Bursa. In particular, three community shelters were examined, but the major number of sera (18 sera) were collected from the largest one that has approximately 100 cats. The age of these 29 cats was not possible to define. Eleven of the 29 cats had a history of severe respiratory signs, otitis and ocular discharge. At the time of sampling all the cats were clinically healthy. All the samples were stored at −20°C prior to analysis. Two monoclonal antibodies (mAb) specific for FCoV I (*SS-FPVTN406-1010-B11F*) and FCoV II (*SS-FPV1146-019E8D*) supplied by Dr Gilles Chappuis (Merial, France) with an IFA titre >1/800 each, were included as controls. Each sample was screened by VN using FCoV II, strain 25/92, propagated on Crandell feline kidney (CrFK) cells.[11] The VN antibody titre was expressed as the reciprocal of the highest serum dilution that completely inhibited viral cytopathic effect. The FCoV II antigen for enzyme-linked immunosorbent assay (ELISA) and Western blotting was prepared as previously described.[12] Briefly, both infected and mock infected cells were harvested 48 h post infection, clarified at $3000 \times g$ for 20 min at 4°C and subsequently, centrifuged for 1 h at $140{,}000 \times g$ at 4°C. The positive pellets had an infectivity titre of $10^{4.50}$ $TCDI_{50}$/50 µl. Microtitre NUNC-immunoplates polysorp (NUNC, A/S, Roskilde, Denmark) were coated with 25 µg/ml of antigen. Each serum, diluted 1:50, was added in duplicate and rabbit anti-cat IgG conjugated to horseradish peroxidase (Labogen, Cortex-Biochem, San Leandro, CA) was employed. Freshly prepared substrate ABTS (Sigma Chemicals, St Louis, MO) was placed into each well and optical density (OD) was determined at 405 nm. The adjusted OD values of each sample were obtained by subtracting the absorbance of the mock antigen-coated well from that of the corresponding virus antigen-coated well. Samples with value exceeding than 0.040 were considered to be positive. FCoV II antigen diluted in Laemmli sample buffer (Biorad, Hercules, CA, USA), final concentration 100 µg/ml, was subjected to electrophoresis in sodium dodecyl sulfate (SDS)-polyacrylamide minigel (5−20%) and transferred on to nitrocellulose membrane Immobilon P (Biorad, Hercules, CA, USA).[12] The membrane was probed with each sample diluted 1:100 and then incubated with rabbit anti-cat IgG conjugated to horseradish peroxidase (Labogen, Cortex-Biochem, San Leandro, CA). For the chromogenic reaction, DAB (Sigma Chemicals, St Louis, MO) was used. The results were then compared using the Cohen's κ test for agreement and repeatability.[13] The κ test is a measure of agreement between two measurements of the same element. Mathematically, κ is defined as agreement beyond change divided by the maximum possible agreement:

$$(AO-AA)/(1-AA)$$

where AO is observed agreement, AA is expected agreement by chance. Values less than 0.4 indicate low agreement, values between 0.40 and 0.75 indicate medium agreement and values greater than 0.75 indicate high agreement between the two rates.

The VN yielded 79 negative and 21 positive sera. The sera were subsequently examined by ELISA which confirmed 74/79 VN-negative sera and the remaining 26 sera as positive. The frequency distribution of positive household cats was compared with that in cattery population and it was observed significantly more seroconverted cats in catteries (18 out of the ELISA-positive sera) than in pet cats. To verify the specificity of the ELISA, the specific mAbs for FCoVs I and II were included. The FCoV I-specific mAb gave negative result (OD = 0.009), while the FCoV II-specific mAb gave positive result (OD = 0.412). The 74 negative sera were also found to be free of FCoVs specific antibodies by Western blotting. The positive samples showed reactivity to the N and M and partially against the S proteins. Using the VN as the gold standard test, ELISA had a relative sensitivity of 100% and a relative specificity of 93.6%, with an overall agreement of 95% (Fig 1). The κ test indicated high agreement between the two tests ($\kappa = 0.86$, 95% CI 0.743−0.980). The positive predictive value (PPV) was 0.8, and the negative predictive value (NPV) was 0.93.[14] The prevalence of FCoV II antibodies in the sampled population based on the gold standard was 21% (95% CI 0.13−0.28). In particular 18/29 cats (95% CI 0.44−0.77) from multi-cat environments (shelters/catteries) were FCoV positive, while 3/71 (95% CI 0.01−0.11) were positive among single cat households.

Feline coronavirus infection is common in multi-cat households. Sera collected at random in California revealed an occurrence of 20%,[15] while in Austria type I virus was found in 62% of the cats tested.[16] Recently, Moestl et al[17] tested samples collected from cats attending clinics and practitioners in Czech Republic

ELISA \ VN	+	-	
+	**21**	5	26
-	0	**74**	74
	21	79	**95**

Fig 1. Assessment of anti-FCoV type II antibodies in cats by ELISA; comparison with the virus neutralisation test taken as the gold standard. Boxes contain numbers of samples.

and Austria. A total of 58% of the Czech cats and 64% of the Austrian cats tested seropositive against FCoV I. Antibody prevalence was reported to be 34% in Australia,[18] and 83% in Switzerland.[19] FCoVs I and II distribution was also studied in southern Italy and a seroprevalence of 72% and 82%, respectively, was detected.[10] In general, antibodies against FCoVs are found in 80–90% of the animals living in catteries and in up to 50% of solitary cats.[20] Based on the gold standard test employed, prevalence of FCoV antibodies in Bursa sampled population is 21%, but differences in the frequency of antibody were found between stray cats (62% of the positive cats) and household cats (4%). The predictivity is a measure of probability closely related to the prevalence of the infection. As the tested cats were healthy at the time of sampling and they live in a region of unknown risk of infection, the prevalence resulted low. Moreover, feline behaviour justifies this result, considering that the high density of population in cattery aids a higher risk of infection and cat-to-cat transmission. The cats were tested once and FCoV seropositivity reflected past exposure. In the 100 sera examined, when the VN was considered as the gold standard test, ELISA had a sensitivity of 100% and a specificity of 93.6%, with an overall agreement of 95%. These data once again confirmed that ELISA is a sensitive and rapid test for the detection of coronavirus antibodies in cats.[10] Considering the cross-reactivity between the two serotypes, ELISA was able to detect antibodies against both, allowing the use of the assay as a reference test for sera screening. Interestingly, the seropositivity observed is low and this apparently strange result is not easy to explain. Therefore, important considerations are necessary. Firstly, our data came from a very small sample of cats collected in a region where the estimated number of cats is not available. Moreover, cats are territorial predators and in the wild the territory is sufficiently vast and extended to feed a community. Domestication modifies the territorial habits only partially, considering that usually the area coincides with human habitation and its surroundings.[21] A further and more important reduction of the territory was imposed by cattery conditions, which has lead to colonial isolated habit of queens, young stock and castrated males. Obviously, the degree of seroconversion within a group may vary on the intensity and frequency of contact between the group members. As previously reported, high antibody titres are more frequently detected in catteries where cats live in crowded conditions.[15,22] The present study submits preliminary data on the epidemiology of FCoV among Turkish cats, and considering that a limited numbers of samples have been handling, more intensive studies and the detection of FCoV I prevalence are obviously necessary to confirm these data.

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